Association of HLA Class I and II Alleles and Extended Haplotypes With Nasopharyngeal Carcinoma in Taiwan

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Background: **Nasopharyngeal carcinoma (NPC), which occurs at a disproportionately high rate among Chinese individuals, is associated with Epstein-Barr virus (EBV). Human leukocyte antigen (HLA) polymorphisms appear to play a role in NPC, because they are essential in the immune response to viruses. We used high-resolution HLA genotyping in a case–control study in Taiwan to systematically evaluate the association between various HLA alleles and NPC.** ***Methods:*** **We matched 366 NPC case patients to 318 control subjects by age, sex, and geographic residence. Participants were interviewed and provided blood samples for genotyping. High-resolution (polymerase chain reaction-based) genotyping of HLA class I (A and B) and II (DRB1, DQA1, DQB1, and DPB1) genes was performed in two phases. In phase I, 210 case patients and 183 control subjects were completely genotyped. In phase II, alleles associated with NPC in the phase I analysis were evaluated in another 156 case patients and 135 control subjects. Extended haplotypes were inferred.** ***Results:*** **We found a consistent association between HLA-A*0207 (common among Chinese but not among Caucasians) and NPC (odds ratio [OR] = 2.3, 95% confidence interval [CI] = 1.5 to 3.5) but not between HLA-A*0201 (most common HLA-A2 allele in Caucasians) and NPC (OR = 0.79, 95% CI = 0.55 to 1.2). Individuals with HLA-B*4601, which is in linkage disequilibrium with HLA-A*0207, had an increased risk for NPC (OR = 1.8, 95% CI = 1.2 to 2.5) as did individuals with HLA-A*0207 and HLA-B*4601 (OR = 2.8, 95% CI = 1.7 to 4.4). Individuals homozygous for HLA-A*1101 had decreased risks for NPC (OR = 0.24, 95% CI = 0.13 to 0.46). The extended haplotype HLA-A*3303-B*5801/2-DRB1*0301-DQB1*0201/2-DPB1*0401, specific to this ethnic group, was associated with a statistically significantly increased risk for NPC (OR = 2.6, 95% CI = 1.1 to 6.4).** ***Conclusions:*** **The restriction of the association of HLA-A2 with NPC to HLA-A*0207 probably explains previously observed associations of HLA-A2 with NPC among Chinese but not Caucasians. The extended haplotypes associated with NPC might, in part, explain the higher rates of NPC in this ethnic group. [J Natl Cancer Inst 2002; 94:1780–9]**

Nasopharyngeal carcinoma (NPC) is associated with the Epstein-Barr virus (EBV) *(1–3).* The incidence of NPC is low throughout most of the world, including Caucasians of North America, but it is common among Chinese in southern China and Southeast Asia. Given the ubiquity of EBV infection worldwide and the relatively infrequent and geographically distinct distribution of NPC incidence, it is widely accepted that factors other than EBV are also important determinants of the risk for NPC. Both environmental (dietary nitrosamines, occupational exposures to wood and formaldehyde, and cigarette smoking) and host genetic (cytochrome P450 2E1 [CYP2E1] gene polymorphisms and polymorphisms in the HLA genes) factors have been implicated *(4–14).*

There is strong *a priori* biologic plausibility for an association between HLA genes and the development of NPC. HLA genes are highly polymorphic and encode for human leukocyte antigen (HLA) molecules that are essential for the presentation of foreign antigens to the immune system, including viral peptides. Class I HLA molecules (e.g., HLA-A and HLA-B) are expressed in all nucleated cells and are involved in the presentation of foreign antigens to cytotoxic $CD8^+$ T cells capable of recognizing and lysing infected cells. Class II HLA molecules (e.g., HLA-DR, HLA-DQ, and HLA-DP) are expressed in a more limited population of cells, most notably cells from the immune system that present foreign antigens to T-helper cells involved in modulating antibody and T-cytotoxic immune responses *(15).* Because nearly all NPC tumors are EBV positive, it is postulated that individuals who inherit HLA alleles with a decreased ability to present EBV antigens to the immune system might be at an increased risk for developing NPC. Conversely, those who inherit HLA alleles that efficiently present EBV antigens to the immune system might be at reduced risk of developing NPC. In fact, studies have confirmed the association between HLA and NPC *(1,2).* Furthermore, these findings are consistent with those showing HLA associations with other virally induced cancers, such as cervical and liver cancers *(16–23).*

Studies of HLA and NPC conducted to date have relied primarily on serologically defined HLA types that provide only low-resolution typing. Each serologic type typically consists of a large number of distinct HLA alleles; for example, the DR4 serotype encompasses more than 30 distinct HLA-DRB1*04 alleles (*0401, *0402, etc.). Studies of serologically defined HLA variants and NPC have shown associations between HLA type

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See "Note" following "References."

and disease that vary across ethnic groups. For example, HLA-A2 alleles have been consistently associated with increased risk of NPC among individuals of Chinese descent but not among non-Chinese populations *(12,24–27).* These apparently discordant findings have shed doubt on the proposed direct link between HLA type and NPC. However, it remains to be determined whether the observed heterogeneity in findings is real or due to different alleles within the A2 serogroup observed in different populations. Other HLA class I serotypes reported to be associated with NPC include A11 and B46 *(25,28,29).* Little is known about the association between class II HLAs and NPC, given the difficulties of typing for class II alleles by serologic techniques *(30–32).*

Polymerase chain reaction (PCR)-based HLA methods are now available that permit high-resolution analysis of polymorphisms at the HLA class I and II loci. We have applied these techniques to a case–control study of 366 case patients with NPC and 318 community control subjects from Taiwan, a country with elevated rates of NPC (approximately seven cases per 100 000 individuals annually compared with fewer than one case per 100 000 Caucasians in the United States). The large size of our study and high-resolution typing enabled a systematic evaluation of HLA alleles and haplotypes associated with the development of NPC.

Materials and Methods

Study Population

Methods for this study have previously been described in detail *(4,8,13,33).* In brief, 378 consecutive case patients newly diagnosed with NPC were identified at their initial visit for a nasopharyngeal biopsy examination from two large referral hospitals in Taipei, Taiwan. Case patients were restricted to individuals younger than 75 years and residents of Taipei city or county for more than 6 months at the time of diagnosis. Case status was confirmed by histologic review.

Individually matched control subjects (1 : 1 ratio) were selected randomly from the Taiwanese National Household Registration system. Control subjects were matched to case patients by sex, age (5-year groupings), and geographic residence. Three hundred seventy-four eligible control subjects were identified. We were unable to match control subjects to four eligible case patients. Informed consent was obtained from all study participants.

Questionnaire Information

All study participants were asked to respond to a detailed risk factor questionnaire that elicited information on sociodemographic characteristics and numerous exposures postulated to be associated with NPC, including cigarette smoking, consumption of nitrosamine-containing foods, and occupation. Dietary data were used to develop an index of dietary nitrosamine consumption, as described *(4).* Complete occupational histories collected from participants were reviewed by an experienced industrial hygienist to assess exposure to wood and formaldehyde, as described *(33).* These factors were evaluated as potential confounders in this analysis because they have been shown to be associated with disease risk in our population *(4,8,13,33).*

Specimen Collection and Testing

All subjects were asked to consent to the collection of blood, and 367 case patients (97.1%) and 321 control subjects (85.8%) agreed. Serum specimens derived from blood were tested for the presence of EBV antibodies (viral capsid antigen immunoglobulin A [IgA], Epstein-Barr nuclear antigen 1 IgA, DNA binding protein IgG, and anti-DNase IgG), as described previously *(33).* DNA was tested for cytochrome P450 2E1 (CYP2E1) genotypes, as described *(13).* DNA was extracted from peripheral blood by use of the QIAamp blood kits (Qiagen, Valencia, CA), according to the manufacturer's instructions.

HLA Genotyping

Extracted DNA was used for HLA genotyping, which was performed in two phases. The first phase of testing involved high-resolution typing of a random sample of 210 case patients and 183 control subjects for HLA-A, HLA-B, HLA-DRB1, HLA-DQA1, HLA-DQB1, and HLA-DPB1 (the sample size of phase I was dictated by financial considerations). Phase I was designed to screen for possible alleles associated with NPC in our population. Alleles identified as being associated with NPC in phase I of our study were then specifically targeted for testing in phase II in an attempt to confirm those associations, and so 156 case patients and 135 control subjects were tested in phase II (three control subjects and one case patient were not tested because their specimens were not available, resulting in 366 case patients and 318 control subjects studied).

In phase I, all samples (from 210 case patients and 183 control subjects) were PCR amplified for the HLA-DRB1, HLA-DQB1, and HLA-DPB1 loci and probed with horseradish peroxidase (HRP)-labeled sequence-specific oligonucleotide probes, as previously described *(34–36).* Group-specific amplifications for subtyping HLA-DR2, HLA-DR3, HLA-DR4, HLA-DR5, HLA-DR6, and HLA-DR8 alleles at the HLA-DRB1 locus were performed, as previously reported *(37,38).* HLA-DRB1-DQB1 haplotypes were inferred from known patterns of linkage disequilibrium for these loci *(37,39–41).* HLA-A and HLA-B high-resolution typing was performed by use of a reverse line-blot typing system by coamplification of the polymorphic exon 2 and exon 3 of each locus with locus-specific biotinylated primers (Dynal Biotech Inc., Lake Success, NY). The resulting amplified sequences (amplicons) were hybridized to arrays of immobilized probes (57 for HLA-A and 83 for HLA-B). HLA-A or HLA-B allele assignment was determined with a computer algorithm of the resulting sequence-specific oligonucleotide probe hybridization patterns.

HLA typing was determined for 203 case patients (96.7%) and 177 control subjects (96.7%) for HLA-A, 205 case patients (97.6%) and 176 control subjects (96.2%) for HLA-B, 196 case patients (93.3%) and 174 control subjects (95.1%) for HLA-DRB1, 203 case patients (96.7%) and 180 control subjects (98.4%) for HLA-DQB1, and 209 case patients (99.5%) and 183 control subjects (100%) for HLA-DPB1.

In phase II, targeted typing was performed by PCR-based dot-blot methods for the following alleles found to be associated with NPC in phase I: HLA-A*0207, HLA-A*1101, HLA-A*3101, HLA-B*13**, HLA-B*4601, HLA-B*5801/2, HLA-DRB1*0301, HLA-DQB1*0201/2, HLA-DQB1*0302, and HLA-DPB1*0401. HLA-B*39** was not included in phase II (despite its association with NPC in phase I) because of the financial and logistical difficulties of screening for this very rare allele (0.49% of case patients and 2.6% of control subjects tested positive for this allele in phase I). HLA-A*0201, although not associated with NPC in phase I, was also targeted in phase II in

an attempt to explain discrepancies observed in previous HLA and NPC studies that used low-resolution serotyping methods. In phase II, we tested 156 case patients and 135 control subjects.

Testing in phase II was performed as described below. Complete HLA-A typing rather than dot-blot screening for individual HLA-A alleles was carried out in phase II typing because of the availability and convenience of the HLA-A reverse line-blot typing system described above.

HLA-DR-DQ typing in phase II was conducted as follows: Samples were coamplified for the HLA-DR-DQ loci by a multiplex amplification (using the same primers as in phase I). The resulting amplicon was dot-blotted onto replicate membranes and probed with a consensus probe for the HLA-DRB1 locus *(34)* and the HLA-DQB1 locus *(35)* to ensure that both loci were amplified in a sample. Samples were then hybridized to detect HLA-DR4 ("VH" epitope—this and other epitope names are in the single-letter amino acid code), HLA-DR3 ("YSTS" and "KGR" epitopes) *(34),* HLA-DQB1*0302 ("LGPPA" epitope), and HLA-DQB1*0201 ("LGLPA" epitope) *(35).* Samples that were positive for VH and LGPPA were listed as containing the HLA-DRB1*04**-DQB1*0302 haplotype. Samples positive for YSTS, KRG, and LGLPA were listed as containing the HLA-DRB1*0301-DQB1*0201/2 haplotype.

Phase II typing for HLA-DPB1 was conducted by amplifying all samples for the DPB1 locus *(36)* and dot-blotting the denatured amplicon onto replicate membranes. All filters were hybridized with a consensus DPB1 probe to ensure amplification *(36).* Filters were then hybridized with probes for the "EEFARF" and "IK" epitopes *(36).* Samples that were positive for both IK and EEARF probes were listed as carrying the HLA-DPB1*0401 allele.

HLA-B phase II typing was conducted by coamplifying exons 2 and 3 as was done in phase I. The resulting amplicon was hybridized to a consensus HLA-B probe for exons 2 and 3 to ensure amplification. The filters were then hybridized to HRP-labeled probes. Probe RAP452B (xACCCAGCTCAAGTGGGA, where x = HRP) recognizes the ITQLKWE epitope in exon 3 of the HLA-B locus and is found in the HLA-B*1301/2 alleles. Probe DB652B (xACCGAGTGAGCCTGCG) recognizes the RVSLR epitope in exon 2 of the HLA-B locus and is found in the HLA-B*4601 allele. These probes were used to detect the presence of their recognized alleles. Samples were listed as carrying the HLA-B*5801/2 allele if they were positive for the following three probes: DB674B, DB703B, and DB758B. DB674B (xGAGGACGGAGCCCCGG) recognizes the PRTEP epitope in exon 2 of the HLA-B locus and is found in the HLA-B*1522, HLA-B*18, HLA-B*35, HLA-B*37, HLA-B*46, HLA-B*51, HLA-B*52, HLA-B*53, and HLA-B*58 alleles. Probe DB703B (xAACATGAAGGCCTCCGC) recognizes the NMKASA epitope in exon 2 and is present in the HLA-B*57, HLA-B*58, HLA-B*1517, and HLA-B*1518 alleles. Probe DB758B (xGG GACGGGGAGACACG) recognizes the WDGET epitope in exon 2 and is found in the HLA-B*57 and HLA-B*58 alleles. Misclassification of HLA-B*5801 could occur if a heterozygous sample contained an HLA-B*57 allele (DB703B and DB758B) and another allele positive for the DB674 probe. However, misclassification is likely to be a rare event, because no samples were found to contain an HLA-B*57 allele in the high-resolution typing conducted during phase I.

Phase II HLA typing was determined on 151 case patients (96.8%) and 134 control subjects (99.3%) for HLA-A, 151 case patients (96.8%) and 133 control subjects (98.5%) for HLA-B, 153 case patients (98.1%) and 133 control subjects (98.5%) for HLA-DRB1, 153 case patients (98.1%) and 133 control subjects (98.5%) for HLA-DQB1, and 154 case patients (98.7%) and 134 control subjects (99.3%) for HLA-DPB1.

Statistical Methods

Allele frequencies were computed and compared between case patients and control subjects with Pearson's χ^2 test or Fisher's exact test (when the number of subjects in a cell was <5) *(42,43).* When case patients and control subjects from phase I were examined, no adjustment for multiple comparisons was made because our plan was to broadly screen for possible associations that would then be confirmed (or not) in phase II. For alleles associated with NPC in phase I ($P<.05$ when allele frequencies were compared and allele frequencies were >0 for case patients and control subjects), odds ratios (ORs) were computed for phase I and phase II separately and for phases I and II combined *(44),* and 95% confidence intervals (CIs) were calculated to determine the statistical significance of the findings. To evaluate the independent effect of HLA on NPC risk, ORs were adjusted for other risk factors for NPC in our population (including the matching factors of age and sex and known risk factors of ethnicity, smoking, cytochrome P450 2E1 polymorphism, and occupational exposure to wood dust and formaldehyde). Unconditional logistic regression methods were used *(44).* Conditional logistic regression was not chosen to avoid loss of information from case patients and control subjects without a matched pair. The ORs presented are adjusted for age, sex, and ethnicity, unless otherwise specified. Stratified analyses were conducted to examine the effect of HLA on NPC within ethnic groups in China and within strata of EBV serology. The joint effects of combinations of two alleles were also evaluated through stratification. Extended haplotype analyses were conducted as follows. HLA-DRB1-DQB1 haplotypes were determined (based on established patterns of linkage disequilibrium) and collapsed into a single locus that we called DRDQ. The patterns of linkage disequilibrium in each of the remaining four loci (HLA-A, HLA-B, HLA-DRDQ, and HLA-DP) were analyzed in a pairwise fashion for sets of three loci each (e.g., linkage disequilibrium for the A-B-DRDQ set was estimated for A-B, B-DRDQ, and A-DRDQ pairs). The normalized linkage disequilibrium parameter D′ expressed the degree of disequilibrium *(45).* Haplotype frequencies were estimated as described *(46,47).* Because complete genotypes were not obtained for all control samples, otherwise identical pairwise estimates for different three-locus sets were made by use of slightly different sized datasets, and as a result, the D′ and *P* values for these pairwise comparisons vary. All statistical tests were two-sided.

Results

Phase I

We tested 210 case patients and 183 control subjects in phase I. Allele frequencies for the HLA-A and HLA-B loci among case patients and control subjects are presented in Table 1. The allele frequency among case patients was statistically significantly higher than that among control subjects for three alleles: HLA-A*0207 ($P = .006$), HLA-B*4601 ($P = .04$), and HLA-B*5801/2 ($P = .01$). It is noteworthy that, among case patients,

Table 1. Phase I: HLA-A and HLA-B allele frequencies among case patients with nasopharyngeal carcinoma (NPC) and control subjects†

	Allele frequencies, %		
	NPC case patients	Control subjects	*P* value‡
HLA-A allele			
0101	0.25	0.57	
0201	14.5	16.4	
0203	6.9	5.1	
0206	3.0	4.8	
0207	**12.8**	**6.8**	**.006**
0209	0.0	0.28	
0210	0.25	0.0	
0301	0.0	1.1	
1101	**29.8**	**37.0**	**.03**
2402	16.5	12.7	
2403	0.0	0.85	
2601	4.2	4.0	
2901	0.25	0.0	
3001	0.25	0.85	
3101	**0.0**	**1.4**	**.02**
3201	0.25	0.57	
3303	11.1	7.1	
7401	0.0	0.57	
HLA-B allele			
07**	0.0	1.1	
0801	0.24	0.0	
13**	**4.1**	**9.4**	**.004**
15**	11.2	15.6	
1802	0.0	0.28	
27**	2.0	3.4	
35**	2.9	1.7	
38**	4.6	3.1	
39**	**0.49**	**2.6**	**.03**
40**	30.5	26.7	
44**	0.24	1.4	
4501	0.24	0.0	
4601	**18.0**	**12.5**	**.04**
48**	1.7	1.1	
51**	6.3	6.0	
52**	1.5	1.1	
5401	2.4	4.5	
5501/2	1.2	1.7	
56**	0.0	0.85	
5701	0.0	0.28	
5801/2	**12.0**	**6.5**	**.01**
6701	0.24	0.0	

†The effective sample size was 2N because each individual contributed two separate alleles. For the HLA-A alleles, 2N = 406 (case patients) and 354 (control subjects); for the HLA-B alleles, 2N = 410 (case patients) and 352 (control subjects).

‡Only *P* values less than .05 from Pearson's χ^2 test or Fisher's exact test (when the number of subjects in a cell was <5) are shown. All statistical tests were two-sided.

Table 2. Phase I: HLA-DRB1-DQA1-DQB1 haplotype frequencies among case patients with nasopharyngeal carcinoma (NPC) and control subjects†

	Haplotype frequency, %		
HLA-DRB1-DQA1-DQB1 haplotype	NPC case patients	Control subjects	*P* value‡
0101-0101-0501	0.79	1.2	
0301-0102-0502	0.0	0.29	
0301-0501-0201/2	**10.6**	**4.7**	**.003**
0401-0301-0301	0.27	0.58	
0401-0301-0302	0.0	0.29	
0403-0301-0302	1.6	3.8	
0404-0301-0302	0.27	1.5	
0405-0301-0301	0.53	0.0	
0405-0301-0302	1.1	0.58	
0405-0301-0401	7.1	7.8	
0405-0301-0402	0.0	0.29	
0406-0301-0302	**2.4**	**5.2**	**.04**
0410-0301-0302	0.27	0.0	
0410-0301-0402	0.0	0.29	
0701-0201-0201/2	1.3	2.6	
0701-0201-0303	0.0	0.29	
0801-0601-0301	0.27	0.0	
0802-0301-0302	0.0	0.29	
0802-0401-0402	0.53	0.58	
0803-0103-0601	13.0	11.0	
0809-0401-0402	0.0	0.29	
0901-0301-0303	18.8	17.4	
1001-0101-0501	0.53	0.87	
1101-0501-0301	7.7	7.0	
1106-0501-0301	0.0	0.29	
1201-0301-0302	0.27	0.29	
1201-0501-0301	2.1	2.9	
1201-0601-0301	0.27	0.0	
1202-0102-0502	0.79	0.58	
1202-0601-0301	7.7	9.6	
1301-0103-0603	0.53	0.29	
1302-0102-0604	0.27	0.58	
1312-0501-0301	1.1	0.0	
1401-0101-0502	2.4	1.7	
1401-0101-0503/1	0.79	0.87	
1403-0501-0301	0.0	0.29	
1404-0101-0503/1	**0.0**	**1.2**	**.05**
1418-0101-0503/1	0.27	0.29	
1501-0102-0502	1.1	0.58	
1501-0102-0601	2.9	3.2	
1501-0102-0602	5.3	2.9	
1501-0102-0603	0.27	0.0	
1502-0101-0501	0.53	1.7	
1502-0101-0502	0.27	0.29	
1502-0102-0502	0.27	0.0	
1602-0102-0502	6.1	5.5	

†The effective sample size was 2N because each individual contributes two separate alleles. For case patients with NPC, 2N = 378; for control subjects, 2N = 344.

‡Only *P* values less than .05 from Pearson's χ^2 or Fisher's exact test (when the number of subjects in a cell was <5) are shown. All statistical tests were two-sided.

the allele frequency was elevated for HLA-A*0207, an A2 allele that is common among individuals of Chinese descent but is rare among individuals of Caucasian descent. Conversely, a comparable allele frequency was observed among case patients and control subjects for HLA-A*0201, one of the most common HLA-A alleles among Caucasians with an allele frequency of more than 25%. The allele frequency among case patients was statistically significantly lower than that among control subjects for four alleles: HLA-A*1101 ($P = .03$), HLA-A*3101 ($P = .02$), HLA-B*13 ($P = .004$), and HLA-B*39 ($P = .03$).

Allele frequencies for HLA-DRB1-DQA1-DQB1 haplotypes among case patients and control subjects are presented in Table 2. Inferred haplotypes rather than individual alleles are presented for these loci because of their close proximity and the resulting strong linkage disequilibrium observed among these three loci. The haplotype frequency among case patients was statistically significantly higher than that among control subjects for only one haplotype, HLA-DRB1*0301-DQA1*0501-DQB1*0201/2 ($P = .003$). The haplotype frequency among case patients was statistically significantly lower than that among control subjects for a single haplotype, HLA-DRB1*0406-DQA1*0301-DQB1*0302 ($P = .04$). When the individual alleles in these two haplotypes were examined, case patients and control subjects were found to be statistically significantly different for HLA-DRB1*0301 ($P = .01$), HLA-DQB1*0201/2 ($P = .02$), and

HLA-DQB1*0302 ($P = .002$) but not for HLA-DQA1*0501 ($P = .09$), HLA-DRB1*0406 ($P = .10$), and HLA-DQA1*0301 ($P = .05$).

Allele frequencies for the HLA-DPB1 locus among case patients and control subjects are presented in Table 3. The allele frequency among case patients was statistically significantly higher than that among control subjects for a single allele: HLA-DPB1*0401 ($P = .004$). None of the alleles examined had a statistically significantly reduced frequency among case patients compared with control subjects.

The following alleles were identified in phase I testing for confirmation in phase II testing: HLA-A*0207, HLA-A*1101, HLA-A*3101, HLA-B*13, HLA-B*39, HLA-B*4601, HLA-B5801/2, HLA-DRB1*0301, HLA-DRB1*0201/2, HLA-DQB1*0302, and HLA-DPB1*0401. HLA-A*0201 was also examined in an attempt to explain the discrepant findings of previous studies based on serologic typing.

Phase II

We tested 156 case patients and 135 control subjects in phase II. For each of the individual alleles examined in phase II, the distribution of alleles among case patients and control subjects and the ORs for disease are presented in Table 4. Combined phase I and phase II results are also presented in Table 4. HLA-A*0207 was associated with increased risk of disease in both phases of our study (combined OR = 2.3, 95% CI = 1.5 to 3.5). Conversely, no increase in risk was observed for HLA-A*0201 in either phase of our study (combined OR = 0.79, 95% CI = 0.55 to 1.2). Other alleles with consistent findings in both study phases include HLA-A*3101 (where no case patients carried this allele) and HLA-B*4601 (combined OR = 1.8, 95% CI = 1.2 to 2.5). For HLA-A*1101, a statistically significant decrease in risk for disease was observed among carriers (OR = 0.64, 95% CI = 0.47 to 0.88). Homozygosity for HLA-A*1101 was observed in approximately 10% of our study population, which allowed us to examine the effect of HLA-A*1101 zygosity on disease risk. A consistent pattern of protection among individuals homozygous for HLA-A*1101 was observed (combined OR = 0.24, 95% CI = 0.13 to 0.46). Less consistent patterns were observed for the other alleles examined.

To reduce the possibility of residual confounding by selected factors, combined phase I and phase II results were examined by restricting the analysis to individuals of Fukkienese descent (approximately 80% of our study subjects) and to individuals seropositive for one or more of the four EBV antibodies measured (approximately 65% of our study subjects). Results observed were similar to those presented in Table 4 (data not shown). Results presented were also not affected by adjustment for cigarette smoking, childhood nitrosamine consumption, occupational wood exposure, occupational exposure to formaldehyde, or cytochrome P450 2E1 genotype, factors previously shown to be associated with NPC in our population *(4,8,13,33)*.

Table 3. Phase I: HLA-DPB1 allele frequencies among case patients with nasopharyngeal carcinoma (NPC) and control subjects†

	HLA-DPB1 allele frequency, %	
HLA-DPB1 allele	NPC case patients (2N = 418)	Control subjects (2N = 366)
0201	14.1	13.9
0202	9.3	7.9
0301	4.8	5.2
0401	10.0	4.6‡
0402	1.4	2.2
0501	47.1	51.1
0901	0.24	0.55
1301	5.5	6.8
1401	1.9	1.6
1501	0.0	0.27
1601	0.0	0.27
1701	0.72	0.82
1901	2.2	0.82
2101	2.2	2.2
2201	0.24	0.0
3801	0.0	0.27
4201	0.0	0.82
4301	0.0	0.27
4801	0.24	0.27

†The effective sample size was 2N because each individual contributes two separate alleles.

‡$P = .004$ for NPC case patients vs. control subjects for HLA-DPB1*0401. None of the P values for the other comparisons in this table was less than .05. P value was obtained from two-sided Pearson's χ^2 test or Fisher's exact test (when the number of subjects in a cell was <5).

Extended Haplotypes

Given the very strong linkage disequilibrium that characterizes the HLA region, the association of an individual allele with disease may reflect linkage disequilibrium with a disease-causing allele at another linked locus. Moreover, some disease-associated alleles at different HLA loci may reflect the disease association of a single extended haplotype that contains these alleles. To examine this issue, we inferred extended haplotypes among the case patients and control subjects in our study population and then evaluated the association between these extended haplotypes and NPC. Estimates of the normalized disequilibrium D′, frequencies, and statistical significance levels are presented in Table 5. Evidence for four extended haplotypes (EHs) was observed among control subjects: HLA-A*3303-B*5801/2-DRB1*0301-DQB1*0201/2-DPB1*0401 (EH1), HLA-A*0203-B*38**-DRB1*1602-DQA1*0102-DQB1*0502-DPB1*1301 (EH2), HLA-A*0207-B*4601-DRB1*0901-DQB1*0303-DPB1*0501 (EH3), and HLA-A*1101-B*40**-DRB1*0405-DQB1*0401-DPB1*0501 (EH4). Among case patients, EH1 and EH2 were observed, but EH3 and EH4 were not observed. In addition, another extended haplotype, HLA-A*2402-B*40**-DRB1*1101-DQB1*0301-DPB1*0501 (EH5), was observed among case patients only.

Interestingly, all of the alleles on haplotype EH1 were increased among the NPC patients in phase I of our study, although the increase in HLA-A*3303, at the telomeric end of the haplotype, was not statistically significant (Tables 1–4). With the exception of HLA-DPB1*1301, all alleles on haplotype EH2 were slightly but not statistically significantly increased among NPC patients in phase I of our study. Our finding that the full EH3 was not observed among case patients is consistent with the observation that only the class I alleles (HLA-A*0207 and HLA-B*4601) on this haplotype were associated with the risk of NPC (Table 1). The finding that EH4 was observed among control subjects only is also consistent with the observation that HLA-A*1101, one of the alleles on this extended haplotype, was associated with a decreased risk for NPC. Finally, EH5, the one extended haplotype identified among case patients only, probably reflects a disease association rather than linkage disequilibrium.

Table 4. Distribution and odds ratios (ORs) with 95% confidence intervals (CIs) for alleles associated with nasopharyngeal carcinoma (NPC) in phase I, phase II, and phases I and II combined

	Phase I			Phase II			Phase I and II combined		
HLA allele	% of case patients	% of control subjects	OR (95% CI)	% of case patients	% of control subjects	OR (95% CI)	% of case patients	% of control subjects	OR (95% CI)
A*0201	28.1	32.2	0.9 (0.55 to 1.4)	8.6	14.2	0.6 (0.3 to 1.3)	19.8	24.4	0.79 (0.55 to 1.2)
A*0207	23.2	13.0	1.9 (1.1 to 3.3)	30.5	14.2	2.9 (1.6 to 5.5)	26.3	13.5	2.3 (1.5 to 3.5)
A*1101†	55.2	59.3	0.84 (0.55 to 1.3)	39.7	59.0	0.46 (0.28 to 0.75)	48.6	59.2	0.64 (0.47 to 0.88)
A*3101	0.0	2.8	NA‡	0.0	6.0	NA	0.0	4.18	NA
B*13**	7.8	15.9	0.43 (0.22 to 0.85)	8.6	11.3	0.81 (0.36 to 1.8)	8.1	13.9	0.58 (0.35 to 0.96)
B*39**	0.98	5.1	0.19 (0.04 to 0.91)	ND	ND		ND	ND	
B*4601	33.2	24.4	1.4 (0.89 to 2.3)	37.8	22.6	2.1 (1.2 to 3.6)	35.1	23.6	1.8 (1.2 to 2.5)
B*5801/2	22.9	12.5	2.0 (1.1 to 3.5)	25.8	22.6	1.1 (0.64 to 1.9)	24.2	16.8	1.5 (1.0 to 2.2)
DRB1*0301	19.9	10.3	2.2 (1.2 to 4.0)	24.2	18.8	1.3 (0.70 to 2.2)	21.8	14.0	1.6 (1.1 to 2.5)
DQB1*0201/2	22.7	13.3	2.0 (1.1 to 3.5)	18.8	24.2	1.3 (0.70 to 2.2)	23.3	15.6	1.6 (1.1 to 2.4)
DQB1*0302	11.3	22.2	0.44 (0.25 to 0.77)	13.1	15.8	0.82 (0.42 to 1.6)	12.1	19.5	0.57 (0.37 to 0.88)
DPB1*0401	17.7	8.7	2.3 (1.2 to 4.4)	16.8	17.2	0.92 (0.49 to 1.7)	17.4	12.3	1.5 (0.97 to 2.3)

†ORs (95% CIs) for individuals heterozygous and homozygous at HLA-A*1101 were as follows: phase I = 1.0 (0.67 to 1.6) and 0.27 (0.12 to 0.62), respectively; phase II = 0.53 (0.32 to 0.86) and 0.21 (0.08 to 0.57), respectively; combined phases I and II = 0.78 (0.56 to 1.1) and 0.24 (0.13 to 0.46), respectively.

‡NA = not applicable; ND = not done.

When case patients and control subjects in phase I of our study (the phase in which complete typing was performed, permitting evaluation) were compared with respect to the four extended haplotypes identified among control subjects, we observed that EH1 was associated with an unadjusted 2.6-fold increased risk of disease (95% CI = 1.1 to 6.4). EH2 and EH3 were associated with unadjusted statistically nonsignificant increases in disease risk (unadjusted OR for EH2 = 1.8 [95% CI = 0.16 to 20] and unadjusted OR for EH3 = 2.8 [95% CI = 0.89 to 9.0]). EH4 was associated with a statistically nonsignificant decreased risk for NPC (unadjusted OR = 0.28, 95% CI = 0.08 to 1.1). Adjustment for age, sex, and ethnicity did not alter these findings. These data indicate that any of the associated class I or class II alleles (or combination of alleles) on EH1 may be responsible for the observed disease association; alternatively, some other locus present on this extended haplotype (EH1) might be responsible.

Joint Effects of Alleles Associated With NPC

For the alleles that were consistently associated with NPC in both phases of our study or in our extended haplotype analysis, we evaluated the joint effect of HLA alleles by a stratification analysis based on combinations of HLA alleles. HLA-A-B-inferred haplotypes were also evaluated. When HLA-A*0207 and HLA-B*4601 (two alleles known to be in linkage disequilibrium) were examined jointly, the effect was strongest for individuals with both alleles (unadjusted OR = 2.8, 95% CI = 1.7 to 4.4), suggesting that both alleles are important determinants (or good markers) of the risk for NPC. For individuals with HLA-A*0207 alone, the unadjusted OR was 1.6 (95% CI = 0.75 to 3.4), and for individuals with HLA-B*4601 alone, the unadjusted OR was 1.2 (95% CI = 0.79 to 1.9). Stratified analysis also suggested that the effect observed for HLA-DPB1*0401 could be explained by HLA-DRB1*0301. The OR observed for individuals with HLA-DRB1*0301 alone (unadjusted OR = 1.6, 95% CI = 0.94 to 2.9) was similar to that observed for individuals with both alleles (unadjusted OR = 1.8, 95% CI = 1.0 to 3.2). Conversely, little effect was seen for individuals with HLA-DPB1*0401 alone (unadjusted OR = 1.1, 95% CI = 0.59 to 2.2).

When HLA-A-B haplotypes were examined, findings consistent with those seen in the single-locus analysis presented in Table 1 were observed (data not shown). Two possible exceptions were the observation that HLA-A*0201-B*51** was associated with an elevation in risk for NPC (OR = 6.4; G statistic = 4.5) and that HLA-A*2402-B*15** was associated with a decreased risk for NPC (OR = 0.2; G statistic = 4.0). The association of HLA-A*0201-B*51** with NPC was not confirmed in a stratified analysis where individuals with both alleles were compared with those with neither allele (OR = 1.3, 95% CI = 0.47 to 3.8), suggesting that this haplotype association, if real, reflects linkage disequilibrium with another locus on chromosome 6. These subgroup effects should be interpreted with caution because of the multiple comparisons made and the small number of case patients and control subjects in some cells. Conversely, the small number of case patients and control subjects in our subgroup analyses might have resulted in real effects being missed.

Discussion

There is now accumulated evidence to suggest that HLA alleles are important in the pathogenesis of virally induced tumors *(12,16–29).* In addition to NPC, other cancers associated with relatively common viruses, such as cervical cancer (caused by human papillomaviruses) and liver cancer (caused by hepatitis viruses), have been associated with HLA polymorphisms. Because the viruses in question are common (EBV, for example, is ubiquitous, and HPV infection occurs in 30% or more of sexually active individuals) but only a small fraction of individuals who are exposed to these viruses are susceptible to tumor development, it is widely accepted that additional factors are necessary for tumor development. Given the central role that HLA plays in the presentation of viral antigens to T cells of the immune system, an important role for HLA in the pathogenesis of these cancers is plausible. It is possible, for example, that individuals who possess HLA alleles capable of inducing a productive immune response (i.e., a cell-mediated immune re-

Table 5. Five-locus haplotypes inferred from two-locus estimates of linkage disequilibrium

Three-locus set	Pairwise comparison†	Frequency	D′	*P* value
Analysis restricted to population control subjects from our Taiwan case–control study				
Extended haplotype 1: A*3303-B*5801/2-DRB1*0301-DQB1*0201/2-DPB1*0401				
A-B-DP	A-B	0.0569	0.86	.001
	A-DP	0.0294	0.64	.001
	B-DP	0.0294	0.64	.001
A-DRDQ-DP	A-DRDQ	0.0376	0.77	.001
	DRDQ-DP	0.0241	0.55	.001
	A-DP	0.0256	0.58	.001
A-B-DRDQ	A-B	0.0430	0.71	.001
	B-DRDQ	0.0469	0.93	.001
	A-DRDQ	0.0400	0.79	.001
B-DRDQ-DP	B-DRDQ	0.0452	0.88	.001
	DRDQ-DP	0.0239	0.47	.001
	B-DP	0.0279	0.47	.001
Extended haplotype 2: A*0203-B*38**-DRB1*1602-DQB1*0502-DPB1*1301				
A-B-DP	A-B	0.0264	0.81	.001
	A-DP	0.0176	0.29	.001
A-DRDQ-DP	A-DRDQ	0.0090	0.12	.050
	DRDQ-DP	0.0211	0.32	.001
	A-DP	0.0151	0.22	.001
A-B-DRDQ	A-B	0.0281	0.81	.001
	B-DRDQ	0.0091	0.22	.010
B-DRDQ-DP	B-DRDQ	0.0077	0.19	.010
	B-DP	0.0094	0.23	.010
	DRDQ-DP	0.0175	0.27	.001
Extended haplotype 3: A*0207-B*4601-DRB1*0901-DQB1*0303-DPB1*0501				
A-B-DP	A-B	0.0433	0.62	.001
A-DRDQ-DP	DRDQ-DP	0.1120	0.23	.050
A-B-DRDQ	A-B	0.0500	0.72	.001
	B-DRDQ	0.0763	0.48	.001
	A-DRDQ	0.0210	0.18	.050
B-DRDQ-DP	B-DRDQ	0.0748	0.48	.001
	DRDQ-DP	0.1109	0.33	.010
Extended haplotype 4: A*1101-B*40**-DRB1*0405-DQB1*0401-DPB1*0501				
A-B-DP	A-DP	0.2314	0.21	.010
	B-DP	0.1785	0.29	.010
	A-B	0.1195	0.11	.001
A-DRDQ-DP	A-DRDQ	0.0545	0.39	.010
	DRDQ-DP	0.0685	0.51	.010
	A-DP	0.2481	0.29	.001
A-B-DRDQ	A-B	0.1268	0.15	.050
B-DRDQ-DP	B-DP	0.1882	0.43	.001
Analysis restricted to case patients from our Taiwan case–control study‡				
Extended haplotype 1: A*3303-B*5801/2-DRB1*0301-DQB1*0201/2-DPB1*0401				
A-B-DP	A-B	0.0851		.001
	B-DP	0.0748		.001
	A-DP	0.0523		.001
A-DRDQ-DP	A-DRDQ	0.0620		.001
	DRDQ-DP	0.0761		.001
	A-DP	0.0513		.001
A-B-DRDQ	A-B	0.0709		.001
	B-DRDQ	0.0859		.001
	A-DRDQ	0.0624		.001
B-DRDQ-DP	B-DRDQ	0.0866		.001
	DRDQ-DP	0.0733		.001
	B-DP	0.0733		.001
Extended haplotype 2: A*0203-B*38**-DRB1*1602-DQB1*0502-DPB1*1301				
A-B-DP	A-B	0.0354		.001
	B-DP	0.0177		.001
	A-DP	0.0152		.001
A-DRDQ-DP	A-DRDQ	0.0163		.001
	A-DP	0.0102		.050
B-DRDQ-DP	B-DRDQ	0.0163		.001
	DRDQ-DP	0.0104		.010
	B-DP	0.0108		.001
A-B-DRDQ	A-B	0.0305		.001
	B-DRDQ	0.0137		.001
	A-DRDQ	0.0109		.010

(Table continues)

Table 5 (continued). Five-locus haplotypes inferred from two-locus estimates of linkage disequilibrium

Three-locus set	Pairwise comparison†	Frequency	D′	*P* value
Extended haplotype 5: A*2402-B*40**-DRB1*1101-DQB1*0301-DPB1*0501				
A-B-DP	A-B	0.0697		.050
	B-DP	0.1704		.050
	A-DP	0.1077		.001
B-DRDQ-DP	B-DRDQ	0.0461		.001
	DRDQ-DP	0.0504		.050
	B-DP	0.1696		.050
A-DRDQ-DP	A-DP	0.1114		.001

†Only pairwise comparisons that displayed statistically significant ($P \leq .05$) degrees of linkage disequilibrium are shown. All statistical tests were two-sided.
‡D′ not presented because linkage disequilibrium cannot be assumed in analysis restricted to case patients.

sponse) to viral antigens are at reduced risk of developing virally induced cancers, whereas those who carry HLA alleles that are not as efficient at mounting a productive immune response to these same viral antigens are more susceptible to these cancers. In this context, it is interesting to note that similar results were obtained in our study when the analysis was restricted to EBV-seropositive individuals. Although this does not negate an important role of HLA alleles in the modulation of T-cell responses to EBV and the subsequent progression to cancer, it does suggest that any role that HLA plays in NPC pathogenesis is independent of the specific patterns of antibody responses seen in individuals who develop NPC.

Results from this study help to clarify previous conflicting reports on the association between the HLA-A2 serogroup and NPC *(12,24–26,48).* Although serology-based studies suggested that HLA-A2 was associated with NPC among individuals of Chinese descent but not among Caucasians, our results demonstrate that these inconsistent observations are likely explained by differences in the distribution of HLA-A2 alleles within the A2 serogroup in different ethnic groups, rather than by a heterogeneous effect of HLA-A2 in different populations *(49).* More specifically, our results suggest that within the A2 serogroup, only the HLA-A*0207 alleles (a genotype common among individuals of Chinese descent but rare among Caucasians) is related to NPC risk, although the HLA-A*0201 allele (a genotype common among Caucasians) is not associated with NPC. Whether HLA-A*0207 is directly involved in NPC pathogenesis or whether it is a marker for another linked gene responsible for NPC development cannot be determined from our data. However, it is interesting that studies *(50–53)* have identified various cytotoxic T-lymphocyte epitopes within the EBV latent membrane proteins (LMP1 and LMP2, proteins that are expressed in a large number of case patients with NPC) and that these epitopes are efficiently presented by HLA-A*0201. Although epitopes within LMP2 were sometimes conserved between different HLA-A*02 alleles, the data suggest that the HLA-A*0207 allele is less efficient than the HLA-A*0201 allele at inducing cytotoxic T-lymphocyte responses to mapped epitopes *(51).*

Another important finding of this study is the identification of extended haplotypes in Chinese individuals that appear to be associated with NPC. Evaluation of linkage disequilibrium within the control subjects in our case–control study revealed four extended haplotypes with evidence of strong linkage disequilibrium, some of which have previously been reported in Asian populations *(54).* The strongest evidence for linkage disequilibrium was shown for HLA-A*3303-B*5801/2-DRB1*0301-DQB1*0201/2-DPB1*0401 (EH1). For EH1, all alleles were individually shown to be associated with NPC. A recent study of the well-characterized Centre d'Etude du Polymorphisme Humain (CEPH) families of European origin *(55)* also provided evidence for the existence of haplotypes that span the 3-megabase interval between HLA-DPB1 and HLA-A. Interestingly, there was no overlap in the extended haplotypes identified in the CEPH families and in our Taiwanese population, confirming the expected ethnic specificity of these extended haplotypes. Although our preliminary findings must be confirmed by other studies, one might speculate that the higher rates of NPC among individuals of Chinese descent are partly explained by the elevated frequency of high-risk alleles and haplotypes.

In addition to HLA-A*0207 and the extended haplotype discussed above, HLA-B*4601 was found to be associated with NPC in both phases of our study. Individuals positive for HLA-B*4601 were at a statistically significant 1.8-fold increased risk of disease. Because HLA-A*0207 and HLA-B*4601 are in linkage disequilibrium, it is difficult to determine whether NPC pathogenesis is associated with one allele, the other allele, both alleles, or a third locus in linkage with HLA-A*0207 and HLA-B*4601. A stratified analysis within our study, although restricted by the small number of individuals with only one of the two alleles, suggests that both alleles are markers of the risk for NPC. The strongest effect (OR = 2.8) was seen among individuals with both HLA-A*0207 and HLA-B*4601. This observation likely explains the association noted between EH3 (HLA-A*0207-B*4601-DRB1*0901-DQB1*0303-DPB1*0501) and the risk for NPC.

Finally, consistent evidence was observed that the HLA-A*1101 allele was associated with a decreased risk for NPC. The effect was most pronounced among individuals homozygous for HLA-A*1101 (OR = 0.24). Support for this association of HLA-A*1101 with the risk for NPC comes from studies *(50,56–58)* that have demonstrated the existence of immunodominant EBV epitopes that are restricted to HLA-A*1101. Thus, a strong and efficient induction of cytotoxic T-lymphocyte responses against EBV-infected cells induced by presentation of EBV epitopes by HLA-A*1101 might explain the decreased risk for NPC associated with HLA-A*1101.

A strength of our analysis was the second phase in which we were able to replicate the effects observed in the initial phase of our study, thus lending credibility to our findings and reducing the need to adjust for multiple comparisons. Curiously, the distribution of some alleles among control subjects varied in the two phases of our study (*see* Table 4). Although the reason for these differences is unclear, it is interesting to note that all but

one of the alleles that varied in frequency between the two phases among control subjects are alleles found in EH1. The facts that frequency differences between phase I and phase II of our study were consistent for alleles in EH1 and that these alleles were examined independently suggest that the differences observed are real and not a result of problems with the methods used to test for HLA in our study. The differences observed might, therefore, have occurred by the chance inclusion of a larger number of individuals who carry EH1 in phase II than in phase I of our study. It should be noted that the allele frequency distribution observed in our study is comparable to that previously reported for Taiwanese populations *(39)*.

In summary, results from our study support a role of specific HLA alleles and extended haplotypes in the development of NPC. Our findings, based on high-resolution HLA genotyping, help explain previously reported inconsistencies *(26)*. Specifically, inconsistencies in the association of HLA-A2 with the risk for NPC reported between populations can now be attributed to differences in A2 genotypes prevalent in various ethnic groups (HLA-A*0207 common among Chinese and associated with NPC; HLA-A*0201 common among Caucasians and not associated with NPC). Our results also support the finding of a decreased risk for NPC associated with HLA-A*1101. The finding of extended haplotypes that are unique to Chinese and that are associated with NPC might partially explain why NPC rates are high in this ethnic group.

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NOTE

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